

Refine Search

Search Results -

Terms	Documents
L6 and reverse near5 orientat\$	0

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L8	Refine Search
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DATE: Thursday, November 03, 2005 [Printable Copy](#) [Create Case](#)

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<u>L8</u>	L6 and reverse near5 orientat\$		0	<u>L8</u>
<u>L7</u>	L6 and reverse		9	<u>L7</u>
<u>L6</u>	L5 and adenovir\$ near5 helper\$		20	<u>L6</u>
<u>L5</u>	L1 and rep52 and rep78 and p5 and p19		20	<u>L5</u>
<u>L4</u>	L3 and ion		1	<u>L4</u>
<u>L3</u>	6491907 [pn]		2	<u>L3</u>
<u>L2</u>	AAV\$ near5 cap near5 (first or second) near5 serotype\$ and AAV\$ near5 rep near5 (first or second) near5 serotype\$		5	<u>L2</u>
<u>L1</u>	AAV\$ near5 cap near5 serotype\$ and AAV\$ near5 rep near5 serotype\$		58	<u>L1</u>

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Inventor Name Search

Enter the first few letters of the Inventor's Last Name.
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Last Name First Name

snyder	richard	<input type="button" value="Search"/>
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byrne	barry	Search
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potter	mark
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loiler	scott	Search
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nash	kevin
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Last logoff: 31oct05 09:18:18
Logon file001 03nov05 15:42:37

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--UPDATED: Important Notice to Freelance Authors--
See HELP FREELANCE for more information

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***Inspec (File 202)
***Physical Education Index (File 138)
***Computer and Information Systems Abstracts (File 56)
***Electronics and Communications Abstracts (File 57)
***Solid State and Superconductivity Abstracts (File 68)
***ANTE: Abstracts in New Technologies (File 60)

RELOADS COMPLETED

*** The 2005 reload of the CLAIMS files (Files 340, 341, 942)
is now available online.

RESUMED UPDATING

***ERIC (File 1)

Chemical Structure Searching now available in Prous Science Drug
Data Report (F452), Prous Science Drugs of the Future (F453), IMS R&D Focus (F445/95
Facts (F390), and Derwent Chemistry Resource (F355).

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>>> of new databases, price changes, etc. <<<

>>>PROFILE is in a suspended state.
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File 1:ERIC 1966-2005/Sep 30
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*File 1: The database is now current with Monthly Updates.

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Cost is in DialUnits
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BEGIN 5,6,55,154,155,156,312,399,BIOTECH,BIOSCI
>>> 135 is unauthorized
>>> 44 is unauthorized
>>> 76 is unauthorized
>>>3 of the specified files are not available
03nov05 15:42:58 User208738 Session D1094.1
\$0.81 0.232 DialUnits File1
\$0.81 Estimated cost File1
\$0.08 INTERNET
\$0.89 Estimated cost this search
\$0.89 Estimated total session cost 0.232 DialUnits

SYSTEM:OS - DIALOG OneSearch
File 5:Biosis Previews(R) 1969-2005/Oct W5
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196507 SEROTYPE?
262714 ADENOVIR?
251434 HELPER?
1558937 REVERSE
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03805299 H.W. WILSON RECORD NUMBER: BGSA98055299 (USE FORMAT 7 FOR
FULLTEXT)
How do animal DNA viruses get to the nucleus?.
Kasamatsu, H
Nakanishi, A
Annual Review of Microbiology v. 52 (1998) p. 627-86
SPECIAL FEATURES: bibl il ISSN: 0066-4227
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 30861

- end of record -

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03051107 H.W. WILSON RECORD NUMBER: BGSI95051107 (USE FORMAT 7 FOR
FULLTEXT)
Viral vectors in gene therapy.
Smith, Alan E

Annual Review of Microbiology (Annu Rev Microbiol) v. 49 ('95) p. 807-38
DOCUMENT TYPE: Feature Article
SPECIAL FEATURES: bibl il ISSN: 0066-4227
LANGUAGE: English
COUNTRY OF PUBLICATION: United States
WORD COUNT: 14692

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00572852

IDENTIFYING NO.: 5P01AG015434-07 0001 AGENCY CODE: CRISP
IMPROVED VIRAL VECTORS FOR GENE TRANSFER TO MUSCLE
PRINCIPAL INVESTIGATOR: CHAMBERLAIN, JEFFREY S
ADDRESS: UNIVERSITY OF MICHIGAN 1301 E CATHERINE STREET ANN ARBOR, MI 48109
PERFORMING ORG.: UNIVERSITY OF MICHIGAN AT ANN ARBOR, ANN ARBOR, MICHIGAN
SPONSORING ORG.: NATIONAL INSTITUTE ON AGING
DATES: 2005/01/98 TO 2004/30/08 FY : 2004

- end of record -

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S2 1 AAV? AND SEROTYPE? AND REVERSE AND ORIENTAT?

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03051107 H.W. WILSON RECORD NUMBER: BGSI95051107 (USE FORMAT 7 FOR FULLTEXT)

Viral vectors in gene therapy.

Smith, Alan E

Annual Review of Microbiology (Annu Rev Microbiol) v. 49 ('95) p. 807-38

DOCUMENT TYPE: Feature Article

SPECIAL FEATURES: bibl il ISSN: 0066-4227

LANGUAGE: English

COUNTRY OF PUBLICATION: United States

WORD COUNT: 14692

- end of record -

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03051107 H.W. WILSON RECORD NUMBER: BGSI95051107 (THIS IS THE FULLTEXT)

Viral vectors in gene therapy.

Smith, Alan E

Annual Review of Microbiology (Annu Rev Microbiol) v. 49 ('95) p. 807-38

DOCUMENT TYPE: Feature Article

SPECIAL FEATURES: bibl il ISSN: 0066-4227

LANGUAGE: English

COUNTRY OF PUBLICATION: United States

RECORD TYPE: Abstract; Fulltext RECORD STATUS: New record

WORD COUNT: 14692

ABSTRACT: The use of DNA as a drug is both appealing and simple in concept. Indeed in many instances the feasibility of such an approach has been established using model systems. In practical terms, however, the delivery of DNA to human tissues presents a wide variety of problems that

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differ with each potential therapeutic application. In this review, the design, production, and application of viral vectors for human gene therapy are considered. Although viral vectors are an obvious starting point because viruses have evolved efficient mechanisms to introduce and express their nucleic acid into recipient cells, by the same token the viral hosts have evolved sophisticated mechanisms to rid themselves of such pathogens. The challenge for the therapeutic use of viral vectors is to achieve efficient and often extended expression of the exogenous gene while evading the host defenses. Methodology used and progress towards that goal are reviewed. Reprinted by permission of the publisher.

TEXT:

INTRODUCTION

Progress in the study of human disorders over the past 25 years has greatly enhanced our ability to describe the molecular basis of many disease states. Molecular genetics techniques have been particularly powerful.

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They have allowed the isolation of the genes associated with common inherited diseases such as cystic fibrosis (CF), as well as the identification of many other genes that contribute to more complex diseases such as cancers. Because our knowledge of the basic mechanisms underlying many biological processes such as gene expression and protein synthesis has also increased, the challenge today is to use all this information in the development of new treatments for disease.

One very logical application is the use of DNA itself as a drug. The delivery of the appropriate gene to a patient with a recessive inherited disease should correct the genetic defect and potentially cure the disease state. Delivery of genes encoding a toxin might kill cancer cells, whereas other genes might be specifically tailored to kill infectious organisms. The list of potential applications is long, and the rationale behind each application is extremely simple.

However, the very simplicity of the theory carries attendant dangers.

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It is easy to overlook the myriad of technical details that must be solved before gene therapy can become a practical therapy capable of treating significant numbers of patients with common diseases. Many problems need to be solved in developing any gene-therapy approach: definition of the cells that constitute the target, entry of DNA into those cells, expression of useful levels of gene product over an appropriate time period, avoidance of the almost inevitable response of the host to the introduced agents, and so on. When first confronted with these challenges, investigators turned to the animal viruses as first-generation agents to deliver DNA to cells. These issues closely parallel those facing a virus in going through its life cycle. Viruses have evolved to be extremely efficient not only at delivering nucleic acid to particular cells but also at evading host-defense mechanisms. Fortunately, viruses themselves have been the object of intense study in the recent past, and much is known of their molecular anatomy. This review summarizes progress in developing viruses as gene-therapy vectors. Rather than attempting to be comprehensive, it

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high-lights practical issues and unanswered questions that remain barriers to the widespread application of gene therapy. Several earlier reviews are also recommended (3, 66, 91, 97, 101, 131).

OVERALL GOALS OF GENE THERAPY

Gene therapy consists of the introduction of nucleic acid into cells of a patient in order to use the expression of that nucleic acid for some therapeutic purpose. Though simple, this definition encompasses an extremely wide range of applications such that defining the goals of all gene-therapy applications in more precise terms is difficult.

The following examples illustrate this diversity: CF is a prototypical monogenic, recessive, genetic disease whose treatment would require delivery, primarily to airway epithelial cells, of an integral membrane protein that functions as a chloride channel. Treatment of solid tumors, for example in brain or lung, could require delivery of a gene encoding a

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toxin or an immune marker or enhancer. A vaccine might need delivery of a gene encoding an immunogen. In some cases, treatment of hemophilia would require delivery of large amounts of a soluble, circulating protein involved in blood clotting, such as factor VIII or IX. Adenosine deaminase (ADA) deficiency treatment requires delivery, ideally to a hemopoietic stem cell, of a gene encoding a housekeeping enzyme.

Bearing in mind these applications, we consider the following issues:

1. What is the nature of the gene product? Is it a nucleic acid, for example an antisense RNA or an RNA decoy? Alternatively, is it a protein?

If so, is it secreted, where is it located, how much is required, and over what time period?

2. What is the target tissue? Is it a particular cell type, and is that cell readily accessible? Does the disease state render it more or less accessible?

3. Is the target cell proliferating or nonproliferating?

4. Is the treatment likely to be in vivo or ex vivo? That is, can

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cells be removed and treated ex vivo, as in all the early protocols, or can, or must, they be treated in situ? If the cells are treated ex vivo, will they be readministered as a separate neo-organ or will they be placed at their normal location? If a neo-organ, will it comprise cells of the same or a different species?

5. Is the requirement for gene expression temporary or permanent? Will the treated cell turn over? Will successful application eliminate the treated cell?

6. Is treatment of all cells in an organ or tissue necessary? Will there be a bystander effect? Will treated cells have a selective advantage?

From these simple considerations, it follows that the requirements for any particular application vary greatly and will profoundly influence the choice of a viral vector to be developed and tested. Factors that will require testing include the efficacy of gene transfer, the efficacy of gene expression, the duration of gene expression, the ability to repeat dosing,

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and the ability to target appropriate cells and avoid inappropriate cells. Confounding factors that may arise include the inability of virus to enter into or integrate into the chromosomes of particular cells, the shutdown of transcriptional promoters, the loss of input DNA, the destruction of treated cells, and the neutralization of input virus or gene product. All of these factors will strongly depend on the choice of viral vector and on the ability of the host to respond to that virus.

CATEGORIES OF VIRUS CONSIDERED

In this review, we consider vector systems based on three different virus groups: retroviruses (90, 92, 93, 137), adenoviruses (11, 12, 52, 129), and adeno-associated viruses (AAV) (17, 76, 102). The more complex herpes virus-based vectors are reviewed elsewhere in this volume (50a). Clearly, viruses can be used as gene-therapy vectors, at least under ideal circumstances. The question that must be answered is, what barriers at present appear likely to limit the practical application of these virus

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vectors?

RETROVIRUSES

Retroviruses comprise a large class of enveloped viruses that contain single-stranded RNA as the viral genome (137). During the normal viral life cycle, viral RNA is reverse transcribed to yield double-stranded DNA that integrates into the host genome and is expressed over extended periods. As a result, infected cells shed virus continuously without apparent harm to the host cell. The viral genome is small (approximately 10 kb), and its prototypical organization is extremely simple, comprising three genes encoding gag, the group specific antigens or core proteins; pol, the reverse transcriptase; and env, the viral envelope protein (Figure 1a). The termini of the RNA genome are called long terminal repeats (LTRs) and include promoter and enhancer activities and sequences involved in integration. The genome also includes a sequence required for packaging

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viral RNA and splice acceptor and donor sites for generation of the separate envelope mRNA (Figure 1b). Importantly, most retroviruses can integrate only into replicating cells, although human immunodeficiency virus (HIV) appears to be an exception (84). This property, possessed by most retroviruses, clearly restricts their use as vectors for gene therapy.

Retroviruses have been studied intensely in the last 20 years, partly because a category of retroviruses causes tumors in animals (137). This ability to cause tumors and to transform the growth properties of cells in culture is now well understood and is based on at least two basic mechanisms. The first is that certain viruses have incorporated activated protooncogenes that upon mutation have acquired the ability to transform cellular growth. The ability of retroviruses to transform cells by this mechanism is not a major concern in gene therapy because the oncogenes are always deleted from the vectors.

The second mechanism of transformation by retroviruses results from

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insertional mutagenesis upon integration of the viral genome. Because the viral LTR has promoter and enhancer activity and is present at both ends of the genome, insertion of an LTR sequence adjacent to a cellular protooncogene can lead to inappropriate expression of a protein involved in cellular regulation. This mechanism was extensively studied in the activation of c-myc by avian leucosis virus (ALV) (61). If retrovirus vectors for gene therapy integrate into the human genome essentially at random, insertional mutagenesis will occur at some frequency. This could lead to protooncogene activation or disruption of a tumor suppressor gene. In practice, however, the frequency of adverse events attributable to insertional mutagenesis is extremely low, perhaps reflecting the observation that much of the human genome is noncoding and that oncogenic transformation usually involves multiple mutagenic events (21, 82).

In essence, retrovirus vectors are relatively simple (66, 92, 93),

containing the 5' and 3' LTRs, a packaging sequence, and a transcription unit composed of the gene or genes of interest (Figure 1c). To grow such a

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vector, one must provide the missing viral functions in trans using a so-called packaging cell line (Figure 1d). Such a cell is engineered to contain integrated copies of gag, pol, and env but to lack a packaging signal so that no helper virus sequences become encapsidated (90).

Additional features added to or removed from the vector and packaging cell line reflect attempts to render the vectors more efficacious or reduce the possibility of contamination by helper virus.

The main advantage of retrovirus vectors is that they integrate and are therefore capable, potentially, of long-term expression. They can be grown in relatively large amounts, but care is needed to ensure the absence of helper virus. The host range of the vectors can be manipulated, but their application is limited to replicating cells. Doubts still remain about the possibility of insertional mutagenesis.

ADENOVIRUSES

Adenoviruses comprise a large class of nonenveloped viruses containing

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linear double-stranded DNA (49, 62, 129). The normal life cycle of the virus does not require dividing cells and involves productive infection in permissive cells during which large amounts of virus accumulates in the nucleus. The productive infection cycle takes about 32-36 hours in cell culture and comprises two phases, the early phase, prior to viral DNA synthesis, and the late phase, during which viral structural proteins and viral DNA are synthesized and assembled into virions. In general, adenovirus infections are associated with mild disease in humans.

The adenovirus genome is much larger (about 35 kb), and its organization is much more complex than retroviruses (Figure 2a) (129). Of the four early transcriptional units, each has a separate promoter and encodes several variously spliced mRNAs. The late transcription unit encodes proteins required for virus assembly. Transcription is somewhat sequential in that the E1 genes are expressed early and in part activate transcription of other early genes. The functions of the early proteins

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fall into various categories; for example, the E1 proteins rescue cells from quiescence, rendering them capable of rapid viral DNA and protein synthesis using predominantly host-coded functions. The E3 genes encode functions to counter host cell defense mechanisms, for example to negate the effects of tumor necrosis factor (TNF) on infected cells and to block

the transport of nascent class 1 proteins of the major histocompatibility complex (MHC) so as to reduce presentation of newly synthesized viral peptides to the host immune system (51). The E2 genes encode proteins involved largely in DNA replication. The E4 region is transcriptionally complex and encodes functions regulating the transition between the early and late phases of the viral life cycle.

Adenoviruses have also been studied intensely over the recent past (129). The reasons are twofold: First, the viral genome comprises several interacting transcription units that are complex enough to be interesting, yet simple enough to allow detailed molecular analysis. As a result, many important concepts in eukaryotic molecular biology were first established

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in studies of adenoviruses, such as mRNA splicing. The second reason is that adenoviruses also can transform the growth properties of cultured cells and form tumors when injected into new-born rodents (129). This process results from adenovirus infection of nonpermissive cells. Under these circumstances, some early gene expression and some DNA synthesis occur, but very little late protein expression and no virus production results. Instead in a very small proportion of the abortively infected cells, viral DNA becomes integrated and early gene expression results. Constitutive expression of Ela and Elb proteins, as in productive infection, negates the tumor-suppressor activity of the Rb and p53 proteins, in this case fortuitously leading to permanent transformation of cellular growth (82). Additional factors are also involved. For example, some serotypes of adenovirus, such as Ad12, are more oncogenic than others, such as Ad2 and Ad5. This ability of adenovirus to transform cells in culture is not regarded as a major problem for gene-therapy applications because despite extensive searches, adenoviruses have never been associated

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with naturally occurring tumors either in humans (54) or animals and because the E1 transforming genes are absent from most defective adenovirus vectors.

Adenovirus vectors are somewhat larger and more complex than retrovirus or AAV vectors (11, 12, 52), partly because only a small fraction of the viral genome is removed from most current vectors. If additional genes were removed, they would need to be provided in trans to produce the vector, which so far has proved difficult. Instead, two general types of adenovirus-based vectors have been studied, E3-deletion and E1-deletion vectors (11, 52). Some viruses in laboratory stocks of wild-type lack the E3 region and can grow in the absence of helper. This ability does not mean that the E3 gene products are not necessary in the wild, only that replication in cultured cells does not require them. Deletion of the E3 region allows insertion of exogenous DNA sequences to yield vectors capable of productive infection and the transient synthesis of relatively large amounts of protein (96, 127).

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Deletion of the E1 region disables the adenovirus, but such vectors can still be grown because there exists an established human cell line (called 293) that contains the E1 region of Ad5 and that constitutively expresses the E1 proteins (53). Most recent gene-therapy applications involving adenovirus have utilized E1 replacement vectors grown in 293 cells.

The main advantages of adenovirus vectors are that they are capable of very efficient episomal gene transfer in a wide range of cells and tissues and that they are easy to grow in large amounts. The main disadvantage is that the host response to the virus appears to limit the duration of expression and the ability to repeat dosing, at least with high doses of first-generation vectors.

ADENO-ASSOCIATED VIRUS

AAV is a very small, very simple, nonautonomous virus containing linear single-stranded DNA (17, 76, 102). The virus requires co-infection with

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adenovirus or certain other viruses in order to replicate. AAV is widespread in the human population, as evidenced by antibodies to the virus, but it is not associated with any known disease.

The genome organization is extremely simple, comprising just two genes--rep, encoding a family of overlapping proteins involved in replication and integration, and cap, encoding a family of three viral structural proteins (Figure 3a). The termini of the genome comprise terminal repeats (TR) of about 145 nucleotides.

Wild-type AAV can integrate its DNA into the host chromosome of cells in the absence of helper virus. This integration seems to occur most often in a specific region of chromosome 19 and involves the rep protein (77).

AAV-based vectors are extremely simple (Figure 3b) (17, 76, 102). They contain only the viral TR sequences flanking the transcription unit of interest. The only constraint appears to be that the length of the vector DNA cannot greatly exceed the viral genome length of 4680 nucleotides.

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Currently, growth of AAV vectors is cumbersome and involves introducing into the host cell not only the vector itself but also a plasmid encoding rep and cap to provide helper functions. The helper plasmid lacks TRs and consequently cannot replicate and package in its own right. In addition, helper virus such as adenovirus is required.

The potential advantage of AAV vectors is that they appear capable of long-term expression in nondividing cells, possibly, though not necessarily, because the viral DNA integrates (46). The vectors are structurally simple, and they may therefore provoke less of a host-cell response than adenovirus. Their main limitation at present is that vectors

are extremely difficult to grow in large amounts.

VECTOR DESIGN

Viral vectors are made up of at least two components, the modified viral genome and the virion structure surrounding it. Most present-generation vectors comprise virion particles based largely on the wild-type virus

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structure. This structure packages and protects the viral nucleic acid and provides the means to bind and enter target cells. However, the viral nucleic acid in a vector designed for gene therapy is changed in many ways. The goals of these changes are to disable growth of the virus in target cells while maintaining its ability to grow in vector form in available packaging or helper cells, to provide space within the viral genome for insertion of exogenous DNA sequences, and to incorporate new sequences that encode and enable appropriate expression of the gene of interest. In this way, we may consider vector nucleic acids as comprising two components: those essential viral sequences that remain and the transcription unit for the exogenous gene.

Because any remaining viral coding sequences might be expressed, even by a disabled viral vector, all nonessential viral sequences should be removed. Thus, ideally, a vector will contain only those viral nucleic acid sequences necessarily required in cis. However, virtually all other

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viral functions are still needed to allow replication of the vector genome for production purposes and to provide proteins required to assemble virion particles. Ideally, a specific packaging or helper cell line would be engineered that expresses those viral functions able to function in trans. In practice, constitutive expression of viral structural proteins and of proteins involved in replication often proves toxic to a cell line, particularly if the life cycle of the virus normally involves acute, productive infection resulting in cell death rather than chronic infection. Thus, appropriate packaging cell lines required to grow many idealized vectors do not exist. The minimal essential features present in current generations of viral vectors are discussed below.

RETROVIRUS VECTORS

ESSENTIAL VIRAL SEQUENCES Because cells tolerate the presence of constitutive retrovirus proteins, the generation of packaging cell lines expressing all the viral proteins is relatively straightforward.

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Consequently, retrovirus vectors need only contain nucleic elements

required in cis. These include the 5' and 3' LTR sequences and the packaging sequence (sometimes referred to as w) that lies downstream of the 5' LTR.

The essential 5' region contains numerous viral functions (Figure 1b), including many involved in nucleic acid replication and integration, promoter and enhancer sequences, a tRNA primer binding site (PBS), and a splice donor site. This region also encompasses the start codons for the gag protein, which in the case of Moloney murine leukemia virus (MoMLV), includes an upstream, in-frame CUG (111) encoding a glycosylated gag protein variant with an N terminal extension as well as the more usual AUG.

LTRs from various retroviruses, including those with specificity for different species, have been used successfully to generate retrovirus vectors (60, 74, 93). Even HIV-based vectors have been produced (109). Because murine retroviruses are well characterized and experiments often

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involve mouse models, vectors commonly utilize the MoMLV LTR and packaging sequence [for example the N2 (5), LNL6 (93), and MFG vectors (105)]. Aside from safety issues involving generation of wild-type recombinant virus in the packing cell line discussed below, for any given application, several features influence the efficacy of an LTR.

Some LTR variants have been especially designed for expression in certain cell types; for example, the murine embryonic stem cell virus (MESV)-based vector is expressed in embryonic cells (55, 56, 60). Changes in the LTR that influence expression include deletions within the U3 region and point mutations that change transcription-factor binding or binding of other cell-specific proteins (56, 72, 130). The PBS immediately adjacent to the 5' LTR also influences expression; for instance, a silencer element from some cells binds to a site for tRNAPro but not tRNAGln (19, 55, 72).

The packaging signal currently used is more extensive than that utilized in first-generation vectors and now includes the N-terminal gag coding sequences (1, 5). Potential expression from the initiation codon

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for gag synthesis rather than the gene of interest can be prevented by mutation of the AUG sequence or insertion of a downstream termination codon (10). Furthermore, the upstream CUG initiation codon in MoMLV is not present in MoMSV. Consequently for applications such as the LNL6 vector, a hybrid packaging sequence is constructed that consists of MoMSV sequences lacking the upstream CUG and MoMLV sequences containing a mutated AUG (93). This region also contains the naturally occurring splice donor upstream of the gag AUG, which normally would interact with the splice acceptor upstream of the env coding sequences (Figure 1a). However, in the absence of the env acceptor site, a cryptic 3' splice acceptor site in the gag coding sequences of the extended packaging signal is activated. The use of the splice acceptor-donor combination appears to be important in some vectors (such as N2) but not in others (LNL6) (93). Essential viral sequences at the 3' end of the viral vector include the LTRs but little else.

TRANSCRIPTION UNIT FOR EXOGENOUS GENE EXPRESSION In the process of

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designing the transcription unit of a retrovirus vector, several questions arise. First, is one protein required or will the vector encode the gene of interest plus a selectable marker? If two proteins are required, will two promoters or a dicistronic mRNA be used? Another important consideration is the amount of the gene product required and the desired duration of expression.

Promoter and enhancer The viral LTR is commonly used as the promoter and enhancer for the gene of interest. Such promoters are usually regarded as relatively strong, and large amounts of gene product can be produced. If a specific cell line is the target, a promoter specially adapted to that cell line might be necessary, as discussed above. When two genes are inserted, a second promoter is often inserted upstream of the second transcription unit (93). A recent systematic study compared different combinations and orientations of the SV40 and CMV promoters with b-galactosidase and neomycin as the test genes and found marked effects, which varied with the precise construct, on both gene expression and vector

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stability (89). An alternative approach is to incorporate internal ribosome entry sites upstream of the second coding region and rely on a single transcript to generate both gene products (73, 83).

An important consideration for applications requiring long-term expression is promoter shut down. Experiments indicate that in some cases, integrated viral DNA persists but transcription is reduced to undetectable levels (29, 107). This finding has led to the search for long-lasting housekeeping promoters or promoters with greater tissue specificity (59, 122). Also, when a secreted protein is the product, the search has been for an alternative tissue in which the gene can be expressed but shutdown may be less problematic (29, 87, 108, 122).

Splicing Because many vectors are constructed by insertion of an appropriate cDNA into a preformed vector cassette, most rely on the splicing signals provided in the vector backbone. In the N2 vector, for example, a splice donor and acceptor are present within the extended w packaging sequence (93). In the MFG vector, the splice acceptor is the

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naturally occurring sequence utilized in production of env mRNA. Placing the AUG of the introduced coding sequence precisely at the site of the env AUG has resulted in highly efficient expression (105).

Conclusions--retrovirus vector design Although the design of retrovirus vectors has reached an advanced stage, it remains largely empirical. This constraint partly results from the very nature of

retroviruses themselves. As the viral nucleic acid is RNA, cryptic splice sites present in any given recombinant genome may be functional and, consequently, preclude the packaging of full-length transcripts. Success can only be assured by testing the vector in appropriate packaging cells to determine whether high titer virus is obtained, and whether the vector is stable. Furthermore, its ability to transduce target cells at high efficiency needs to be tested.

In this regard, surprisingly few systematic studies have been done to compare the properties of related vectors. Often constructs used in comparative studies differ by more than one feature, making interpretation

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